



US009636362B2

(12) **United States Patent**  
**Gurtner et al.**

(10) **Patent No.:** **US 9,636,362 B2**  
(45) **Date of Patent:** **May 2, 2017**

(54) **PULLULAN REGENERATIVE MATRIX**

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(\*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 472 days.

(21) Appl. No.: **12/932,736**

(22) Filed: **Mar. 3, 2011**

(65) **Prior Publication Data**

US 2011/0305745 A1 Dec. 15, 2011

#### **Related U.S. Application Data**

(60) Provisional application No. 61/339,559, filed on Mar. 4, 2010.

(51) **Int. Cl.**

**A61K 38/39** (2006.01)  
**A61K 8/73** (2006.01)  
**A61K 35/28** (2015.01)  
**A61L 15/22** (2006.01)  
**A61L 15/40** (2006.01)  
**A61L 15/42** (2006.01)  
**A61L 15/44** (2006.01)  
**A61L 15/60** (2006.01)  
**A61L 27/26** (2006.01)  
**A61L 27/38** (2006.01)  
**A61L 27/48** (2006.01)  
**A61L 27/52** (2006.01)  
**A61L 27/54** (2006.01)  
**A61L 27/56** (2006.01)  
**A61K 9/70** (2006.01)

(52) **U.S. Cl.**

CPC ..... **A61K 35/28** (2013.01); **A61K 8/732** (2013.01); **A61K 38/39** (2013.01); **A61L 15/225** (2013.01); **A61L 15/40** (2013.01); **A61L 15/425** (2013.01); **A61L 15/44** (2013.01); **A61L 15/60** (2013.01); **A61L 27/26** (2013.01); **A61L 27/3804** (2013.01); **A61L 27/48** (2013.01); **A61L 27/52** (2013.01); **A61L 27/54** (2013.01); **A61L 27/56** (2013.01); **A61K 9/7007** (2013.01); **A61L 2300/404** (2013.01); **A61L 2300/414** (2013.01)

(58) **Field of Classification Search**

CPC ..... **A61K 35/28**  
See application file for complete search history.

(56) **References Cited**

#### **U.S. PATENT DOCUMENTS**

4,841,962 A \* 6/1989 Berg et al. .... 602/50  
2010/0221301 A1 \* 9/2010 Le Visage et al. .... 424/422

#### **FOREIGN PATENT DOCUMENTS**

WO WO-2007124023 A2 \* 11/2007 ..... C21N 5/08

#### **OTHER PUBLICATIONS**

Abed; et al., "A Biocompatible Polysaccharide Hydrogel—Embedded Polypropylene Mesh for Enhanced Tissue Integration in Rats", *Tissue Engineering: Part A* (2008), 14(4):519-527.

Autissier; et al., "Pullulan-based hydrogel for smooth muscle cell culture", *Journal of Biomedical Materials Research Part A* (2007), 82(2):336-342.

Badiavas; et al., "Participation of Bone Marrow Derived Cells in Cutaneous Wound Healing", *Journal of Cellular Physiology, Journal of Cellular Physiology* (2003), 196:245-250.

Kataoka; et al., "Participation of Adult Mouse Bone Marrow Cells in Reconstitution of Skin", *American Journal of Pathology* (2003), 163(4):1227-1231.

Kato; et al., "Nanogel-Based Delivery System Enhances PGE2 Effects on Bone Formation", *Journal of Cellular Biochemistry* (2007), 101:1063-1070.

Lataillade; et al., "New approach to radiation burn treatment by dosimetry-guided surgery combined with autologous mesenchymal stem cell therapy", *Regen. Med.* (2007), 2(5):785-794.

Shimizu; et al., "Nanogel DDS enables sustained release of IL-12 for tumor immunotherapy", *Biochemical and Biophysical Research Communications* (2008), 367(2):330-5.

Thebaud; et al., "Human endothelial progenitor cell attachment to polysaccharide-based hydrogels: a pre-requisite for vascular tissue engineering", *J Mater Sci: Mater Med* (2007), 18(2):339-345.

Wu; et al., "Mesenchymal Stem Cells Enhance Wound Healing Through Differentiation and Angiogenesis", *Stem Cells* (2007), 25(10): 2648-59.

\* cited by examiner

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(57) **ABSTRACT**

Compositions and methods are provided for the manufacture and use of a pullulan-based collagen hydrogel film with controlled porosity. The hydrogel is fabricated with salt-induced phase inversion and cross-linking to form a reticular scaffold. This soft collagen scaffold displays excellent handling characteristics, durability, and a porous dermal-like ultrastructure that is maintained in vitro. Cells, including cells involved in tissue repair, are viably sustained within the scaffold. The hydrogel films are biodegradable, and find particular use in wound healing, where the hydrogel scaffold can be replaced by dermal cells over time.

**22 Claims, 12 Drawing Sheets**